TO THE EDITOR:

Significance of antinuclear antibodies in primary immune thrombocytopenia: results of the CARMEN registry

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In a recent issue of *Blood Advances*, Hollenhorst et al provided new insight on the significance of antinuclear antibodies (ANAs) in adult patients with immune thrombocytopenia (ITP).[1] In this retrospective monocentric series, 144 patients tested for ANAs between 1992 and 2015 were included. The prevalence of ANAs (threshold used for positivity, 1/40) was 65%. The presence of ANAs was associated with a higher risk for thrombosis.[1]

The utility of ANA dosage in primary ITP as regards patients' characteristics, risk for ITP chronicity, response to treatment, and development of systemic lupus erythematosus (SLE) is debated (Table 1).[2-7] The presence of ANAs has been associated with a 50% response rate of ITP to hydroxychloroquine.[8] Of note, 2019 international guidelines for ITP management stated that ANAs might be associated to chronicity; as a consequence, these guidelines listed ANAs among the "tests of potential utility in the management of an ITP patient."[9] In contrast, 2011 and 2019 American Society of Hematology guidelines do not recommend systematic ANA testing.[10,11]

Because of these discrepancies, we aimed at assessing the association of ANAs with several outcomes, including ITP presentation, response to first-line treatment, thrombosis, and SLE occurrence in the French prospective multicenter CARMEN registry. We have previously shown in this cohort that ANA positivity is associated with chronic evolution of ITP.[2]

The CARMEN (Cytopénies Auto-immunes Registre Midi-Pyrénéen) registry is a multicenter prospective registry aimed at following all adults with newly diagnosed ITP in the Midi-Pyrénées region in the southwest of France (3 million inhabitants) since June 2013. Ethical approval was obtained from the French Data Protection Authority (Commission Nationale de l'Informatique et des Libertés–CNIL) for the CARMEN registry, authorization numbered 2012-438. Inclusion criteria in the registry are: age 18 years or older, incident ITP (diagnosis <3 months) according to international definition (platelet count <100 × 10^9/L and exclusion of other causes of thrombocytopenia),[12] follow-up in the region, and no opposition to data recording. ANAs were tested at ITP diagnosis according to French guidelines,[13,14] and recorded in the database. Positivity is defined in the registry by titer at least 1/160.[15]

For the present study, we selected all patients with primary ITP included in the CARMEN registry from 1 June 2013 to 31 December 2017. We assessed the association of ANA with age at ITP diagnosis, sex, history of other autoimmune disease, presence of bleeding at ITP diagnosis, overall, and by categories (cutaneous bleeding, mucosal bleeding, and serious bleeding, defined by intracranial, gastrointestinal bleeding, or macroscopic hematuria), platelet count at ITP diagnosis, response to first-line treatment, and occurrence of thrombosis and of SLE after ITP diagnosis (end of follow-up, 31 December 2019). Comparison tests were the χ^2 or the Fisher's exact tests for binary variables and the Wilcoxon Mann-Whitney test for quantitative variables. The occurrence of thrombosis over time from ITP diagnosis was assessed using Kaplan-Meier curves and log-rank tests. Analyses were carried out using SAS V9.4 (SAS Institute, Cary, NC).

Overall, 278 adult patients with incident primary ITP were prospectively included in the CARMEN registry during the study period. Among them, 215 were tested for ANAs (77.3%), described in Table 2. Median age was 64.0 years, and 107 patients (49.8%) were men. The median platelet count at ITP diagnosis was 18 × 10^9/L, and 126 patients (58.6%) had bleeding symptoms at ITP diagnosis. Overall, 170 patients (79.1%) were treated for ITP. First-line treatment consisted of corticosteroids alone in

Table 1. Previous studies assessing association of ANAs with various outcomes in ITP patients

Study	Number of tested patients	Design	Threshold for ANA positivity	Prevalence, %	Outcomes
Kurata et al[3]	66 chronic ITP	Retrospective	1/40	44	No SLE and Sjögren syndrome during follow-up in the ANA⁺ group
Vantelon et al[4]	122 chronic ITP	Retrospective	1/40	13	2 SLEs during follow-up in the ANA⁺ group
Altintas et al[5]	108 newly diagnosed and chronic ITP	Retrospective	1/80	33.6	No difference regarding chronic evolution
Abbasi et al[6]	46 newly diagnosed ITP	Retrospective	1/40	21.7	No difference regarding age, history of autoimmune disease, platelet count at diagnosis
					Response to corticosteroids less frequent if ANA⁺
Grimaldi-Bensouda et al[7]	136 newly diagnosed ITP	Prospective	1/80	25.7	No difference regarding age, sex, platelet count at diagnosis, chronic evolution
					More frequent familial history of autoimmune disease and less frequent bleeding at diagnosis if ANA⁺
Moulis et al[2]	85 newly diagnosed ITP	Prospective	1/160	44.7	Risk for chronicity if ANA⁺ (OR, 2.89; 95% CI, 1.08-7.74)
Hollenhorst et al[1]	144 ITP	Retrospective	1/40	65	Higher risk for thrombosis if ANA⁺
					No difference in remission at last follow-up

CI, confidence interval; OR, odds ratio.

85 patients, corticosteroids plus IV immunoglobulin (IVIg) in 78, IVIg alone in 2, romiplostim in 1, eltrombopag in 1, corticosteroids plus IVIg and eltrombopag in 1, corticosteroids plus IVIg and romiplostim in 1, and corticosteroids plus IVIg and vinblastine in 1.

Among the 215 patients tested for ANAs, 92 (42.8%) were positive with a titer of at least 1/160 (titer ≥1/320, n = 48; ≥1/640, n = 26; ≥1/1280, n = 13; specificity was anti-SSA [Sjögren's syndrome–related antigen A] antibodies in 10 patients, anti-SSB [Sjögren's syndrome–related antigen B] in 5, anti-centromere in 3, anti-DNA in 2, anti-Sm/RNP [Smith/ribonucleoprotein] in 2, anti-Sm in 1, anti-RNP in 1, anti-Scl70 in 1). Results of a comparison of variables between patients with positive ANAs and those with negative ANAs are indicated in Table 2. Overall, no difference was observed for any outcome except a slightly higher median age in the ANA-positive group. During follow-up, 16 thromboses occurred in 14 patients: 10

venous events (including 2 in the same patient) and 6 arterial events. There was a trend toward a slightly increased occurrence of thrombosis in the ANA-positive group (8.7% vs 5.4%). Kaplan-Meier curves showed no difference in the risk for thrombosis over time between groups during the first 3 years after ITP diagnosis (supplemental Figures 1-3). The incidence of thrombosis differed between groups thereafter, but with few patients at risk. Of note, 9 patients who developed thrombosis were tested for antiphospholipid antibodies (anticardiolipin antibodies, n = 9; lupus anticoagulant, n = 5; anti-β2-GP-I antibodies, n = 6), and none was positive. No SLE occurred during follow-up.

The prevalence of ANAs in this prospective study is close to the prevalence found by Hollenhorst et al, using the threshold of at least 1/160 (42.8% vs 41%, respectively). The authors did not assess the association of ANAs with age, but as in our study, there was no

Table 2. Association of ANA positivity (≥1/160) with various outcomes in the CARMEN registry (215 tested patients)

Outcomes	Total (N = 215)	Positive ANAs (n = 92)	Negative ANAs (n = 123)	p
Age, median (Q1-Q3), y	64.0 (41.0-79.0)	66.0 (44.0-83.5)	60.0 (39.0-76.0)	.07
Male sex, n (%)	107 (49.8)	44 (47.8)	63 (51.2)	.6
History of autoimmune disease, n (%)	34 (15.8)	17 (18.5)	17 (13.8)	.3
Bleeding at ITP diagnosis, overall, n (%)	126 (58.6)	53 (57.6)	73 (59.4)	.8
Cutaneous bleeding at ITP diagnosis, n (%)	112 (52.1)	47 (51.1)	65 (52.8)	.8
Mucosal bleeding at ITP diagnosis, n (%)	56 (26.1)	23 (25.0)	33 (26.8)	.8
Serious bleeding at ITP diagnosis, n (%)*	13 (6.1)	7 (7.6)	6 (4.9)	.4
Platelet count at ITP diagnosis, median (Q1-Q3), ×10⁹/L	18.0 (5.0-50.0)	18.0 (5.0-49.5)	18.0 (6.0-51.0)	.9
Response to first-line treatment, n (%)	136 (80.0)	62 (83.8)	74 (77.1)	.3
Complete response to first-line treatment, n (%)	101 (59.8)	49 (67.1)	52 (54.2)	.2
Thrombosis during follow-up, n (%)	14 (6.5)	8 (8.7)	6 (4.9)	.3
Venous thrombosis during follow-up, n (%)	9 (4.2)	5 (5.4)	4 (3.2)	.5
Arterial thrombosis during follow-up, n (%)	6 (2.8)	4 (4.3)	2 (1.6)	.4

*Intracranial, gastrointestinal bleeding, or macroscopic hematuria.

difference by sex. Their main finding was the association with thrombosis occurrence: no event was observed in ANA-negative patients, whereas 17 of the 19 patients who experienced thrombosis were ANA-positive. In contrast, we found a slight and delayed increased frequency of thrombosis in the ANA-positive group. The follow-up was longer in the retrospective study by Hollenhorst et al (study period, 1992-2015), and the titer threshold for ANA positivity was higher in our study, which may explain in part the discrepancies between the 2 cohorts. Moreover, the trend we observed was explained by delayed occurrence of thrombosis, with very few patients at risk: a single event in 1 group by chance could be responsible for a higher difference between groups. Altogether, the trend we observed of a slight and delayed increased risk for thrombosis in ANA-positive patients must be taken with caution. Of note, this possible association seems independent of the presence of antiphospholipid antibodies, and particularly of lupus anticoagulant, in our study, as in the study by Hollenhorst et al (among 19 thrombotic events, 17 were ANA-positive, including 4 only with lupus anticoagulant),[1] which has been demonstrated as strongly associated with thrombosis in ITP.[16] The absence of development of SLE over time is in line with findings by Kurata et al, with a 3-year follow-up.[3]

The CARMEN registry is fully prospective, multicenter, and aimed at completeness of case recording in the region. Epidemiology of ITP in the CARMEN registry is representative of ITP epidemiology in France.[2,17] Only incident cases are recorded. The main limitation of this study, as a result of the real-life design of the registry, is that not all patients were tested for ANAs. However, 215 patients were tested, and this is to date the largest cohort of patients with ITP tested for ANAs (Table 1). Last, we could not assess the association of lower titers of ANAs with outcomes because the titer of 1/160 is the definition of ANA positivity used in the registry as a result of the frequency of low titers in the general population[15]; this may have affected the classification of patients compared with the series by Hollenhorst et al, as described earlier.

Overall, our study suggests that the presence of ANAs at titer at least 1/160 were not associated with ITP presentation, response to first-line treatment, significant occurrence of thrombosis during the first years after ITP diagnosis, and development of SLE during follow-up.

Contribution: G.M. designed the study, performed statistical analyses, and wrote the paper; G.M., T.C., and J.G. acquired the data; G.M. and J.G. carried out the data management; and all the authors critically reviewed the manuscript and gave final approval to the submission.

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A complete list of the members of the CARMEN investigators group appears in "Appendix."

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